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# EVALUATION OF ACUTE TOXICITY OF THIAMETHOXAM IN ALGERIAN HONEYBEE *APIS MELLIFERA INTERMISSA* AND *APIS MELLIFERA SAHARIENSIS*

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## ABSTRACT

Thiamethoxam [3-(2-chlorine-1, 3-thiazole-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro) amine], a systemic insecticide of the group of neonicotinoids with a large spectrum of action at low concentrations. It is used in the control of sucking insects and some chewing species, because of its excellent absorption and translocation in plants. The acute toxicity of contact and oral applications on two *Apis mellifera* subspecies, *Apis mellifera intermissa* and *Apis mellifera sahariensis* was investigated. In all toxicological studies, each dose included three cages of 20 individuals and each study was replicated three times. The dose–mortality relation revealed directly proportional relationship between the administered dose of thiamethoxam and mortality observed. The mortality is reached maximum at 24 hours after treatment with doses above 50 ng / bee after oral application. Response kinetics showed classic kinetics. The higher the dose of thiamethoxam and faster is high mortality appears. After oral intoxication, the LD50 values of thiamethoxam at 24h were about 12,3 ng/bee for *A. m. intermissa* and 13.3 ng/bee for *A. m. sahariensis.* After contact application, the LD50 values at 24 h were approximately 26 ng/bee for *A. m. intermissa and* 43.3 ng/bee for *A. m. sahariensis.*

**KEYWORDS:** Thiamethoxam, *Apis mellifera intermissa*, *Apis mellifera sahariensis*, Honeybees, Lethal Dose, Acute Toxicity

## INTRODUCTION

The place of bees in the environment has many aspects; agronomic, economic, ecological and scientific. It plays an important economic role as a carrier of beekeeping (honey, royal jelly, pollen, propolis and wax) and agriculture by providing a quantitative and qualitative increase crop (Vaissiere 2002; Haubruge *et al*., 2006; Breeze *et al*, 2011). With its complex social behavior, the honeybee is one of the best scientific models to study the learning functions, memory and orientation, particularly in the activity of chiseling. In addition, an ecological point of view, this is a useful insect bio- indicator of high environmental sensitivity because it is in contact with pollutants from various sources (Kevan, 1999). Bees can get in contact with pesticides when foraging in treated crops. Of weakening phenomena apiaries with a decrease in activity without the observation of pathogens (Faucon & Colin, 1983). In Algeria, for ten years, beekeepers observed serious disturbances in their colonies and highlight the responsibility of some insecticides used in crop protection. Indeed, many beekeepers indicate a weakening or even a total depopulation of the hive.

This may be due to alterations in the nervous system of bees, especially since 90% of the insecticides used in agricultural and forest areas have neurotoxic properties. Thiamethoxam is the first representative of the second generation neonicotinoid and belongs to the subclass of thianicotinyls (Maienfisch *et al*., 1999). It has exceptional systemic features

and a strong preventive effect against the transmission of the virus. Thiamethoxam show long-lasting residual activity (Maienfisch *et al*., 2001).

The objective of this study was to evaluate of the acute effects of thiamethoxam to honeybee workers. Its intrinsic toxicity was studied by determining the laboratory-based median lethal dose (LD) after oral and contact applications in local honeybees Apis *mellifera intermissa* Buttel-Reepen, 1906 and *Apis mellifera sahariensis* Baldensperger, 1924. Mortality kinetics were also studied using different thiamethoxam doses. The acute toxicity of insecticides allows the determination of a sublethal level. This sublethal level is important to study the chronic toxicity of insecticides and their adverse sublethal effects than can induce a deleterious impact on honeybee populations.

## MATERIALS AND METHODS

### Materials

Thiamethoxam is marketed under the brand Actara for foliar and soil and Cruiser seed treatment (Maienfisch *et al*., 2001). It is 99.7% purity and was obtained from the office Syngenta Algeria. For each subspecies, bee workers (*A. m. intermissa* and *A. m. Sahariensis*) were captured from honey and pollen combs in a same healthy queen-right colony for all bio assays; all drones were discarded. Immediately before treatment, bees were anesthetized with carbon dioxide and kept in cages (10, 5 X 7, 5 X11, 5 cm) in a temperature-controlled chamber at 25±2°C with 60 ±10% relative humidity. Bees were fed with candy and water ad libitum (EPPO, 1992).

### Experimental Conditions

In each experiment, three cages of 20 bees were used for each dose of treatment. Experiments were replicated at least three times; control mortality was less than 15% in all experiments (EPPO, 1993).

### Modes of Treatment

**Oral Application:** The honeybees were deprived of food for 2h before administration of thiamethoxam. Thiamethoxam solutions were prepared in a 1% acetone solution and then diluted 10-fold in the 50% (w/v) feeding sucrose solution. The final concentration of acetone solution in the sucrose solutions of control and assay tests was 0.1% (v/v). The dosing solutions were prepared fresh for each test. Each bee received 10 µl of 50% sucrose solution (vehicle) containing graded doses of thiamethoxam or the dosing vehicle alone (control). After consuming this solution, bees were fed with candy and water ad libitum. Mortality was recorded at 24, 48 and 72 h.

**Contact Application:** One microliter of insecticide solution in 100% acetone solution (vehicle) was applied with a microsyringe on the dorsal thorax. After the application, all bees were fed with candy and water ad libitum. Control bees received 1 µl of the vehicle. Bee mortality was recorded 24, 48 and 72 h after topical application.

### Data Analysis

Mortality data were corrected according to Abbott (1925). The LD50 values are obtained by probit transformation of mortality rates. One-way analysis of variance was used to evaluate differences between groups.

## RESULTS

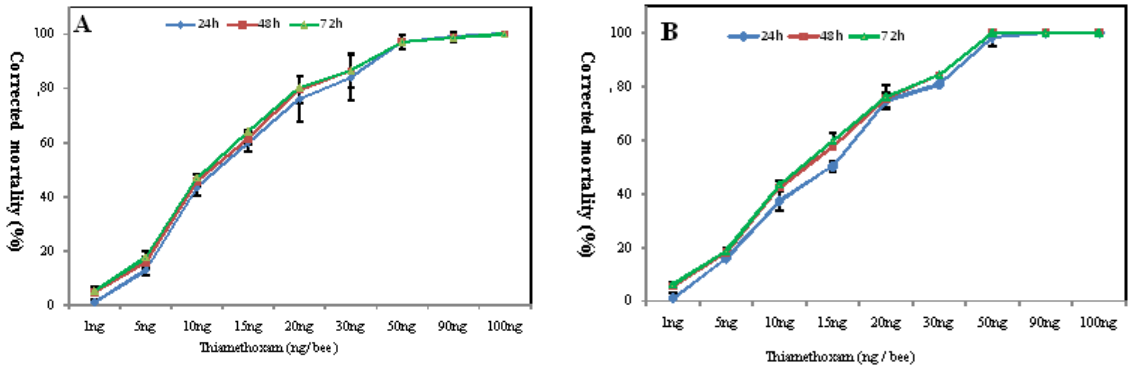
### Observation of Symptoms Induced by Thiamethoxam

The toxicity of thiamethoxam to bee workers was investigated with different application modes. In all

toxicological studies, each dose included three cages of 20 individuals and each study was replicated three times. Both *A. m. intermissa* and *A. m. sahariensis* exhibited neurotoxic symptoms such as trembling, tumbling, and lack of coordination within 24 h of thiamethoxam exposure. We also noted the appearance of the first cases of mortality, already 15 minutes after ingestion of high doses of toxic by both honeybees subspecies.

**Oral Toxicity**

The results of acute oral toxicity in *A. m. intermissa* and *A. m. sahariensis* are shown in Figure 1 A and B. The character increases for the two both subspecies according to the administered dose. Indeed, there is a directly proportional relationship between the administered dose of thiamethoxam and mortality observed. The mortality is reached maximum at 24 hours after treatment with doses above 50 ng / bee mortality maximum is reached after 48 hours (Figure 1). For doses between 15 and 20 ng / bee, reported mortality rates exceed 50% for the two races of bees. But 100% mortality are achieved with a dose of 100 ng / bee for *A. m. intermissa* after 24 hours and with a dose of 50 ng / bee for *A. m. sahariensis* after 48 hours of treatment. Doses of 5 and 10 ng / bee generate mortality in the population of *A. m. intermissa* development experience 13% and 43% after 24 hours. Similarly to *A. m. sahariensis* at doses of 5 and 10 ng / bee mortality rates are generated by 16% and 37 % after 24 hours (Figure 1B). Small variations in terms of mortality between repetitions are observed as evidenced by the low standard deviations. Indeed, it should be noted that for some experiments the maximum mortality is reached after 24 hours of treatment. Thus the dose - mortality curves are superimposed relationship. The results of ANOVA analysis ($p < 0.0001$) show that the treatment effect is highly significant in terms of the sensitivity for thiamethoxam *A. m. intermissa* and *A. m. sahariensis.*




**A:** *Apis mellifera intermissa*; **B:** *Apis mellifera sahariensis*. Bee Mortality Observed 24, 48 h and 72 h after Oral Application of Different Thiamethoxam Doses. Data Represented the Means ± SD of Three Experiments Performed in Triplicate. The Absence of Error Bars Corresponds to SD = 0

**Figure 1: Dose Response Relation Resulting from Oral Exposure to Thiamethoxam**

**Kinetics of Mortality**

The kinetics of mortality are shown in Figure 2 A and B. The aspect of this is classic kinetics. The higher the dose of thiamethoxam and faster is high mortality appears. For *A. m. intermissa*, the maximum mortality is reached 24 hours after ingestion of thiamethoxam to between 30 and 100 ng / bee (Figure 2A). For *A. m. sahariensis*, 100% mortality are achieved after 24 hours at doses of 90 and 100 ng / bee for when doses between 20 and 50 ng / bee mortality maximum occurs 48 hours after ingestion of thiamethoxam (Figure 2B).

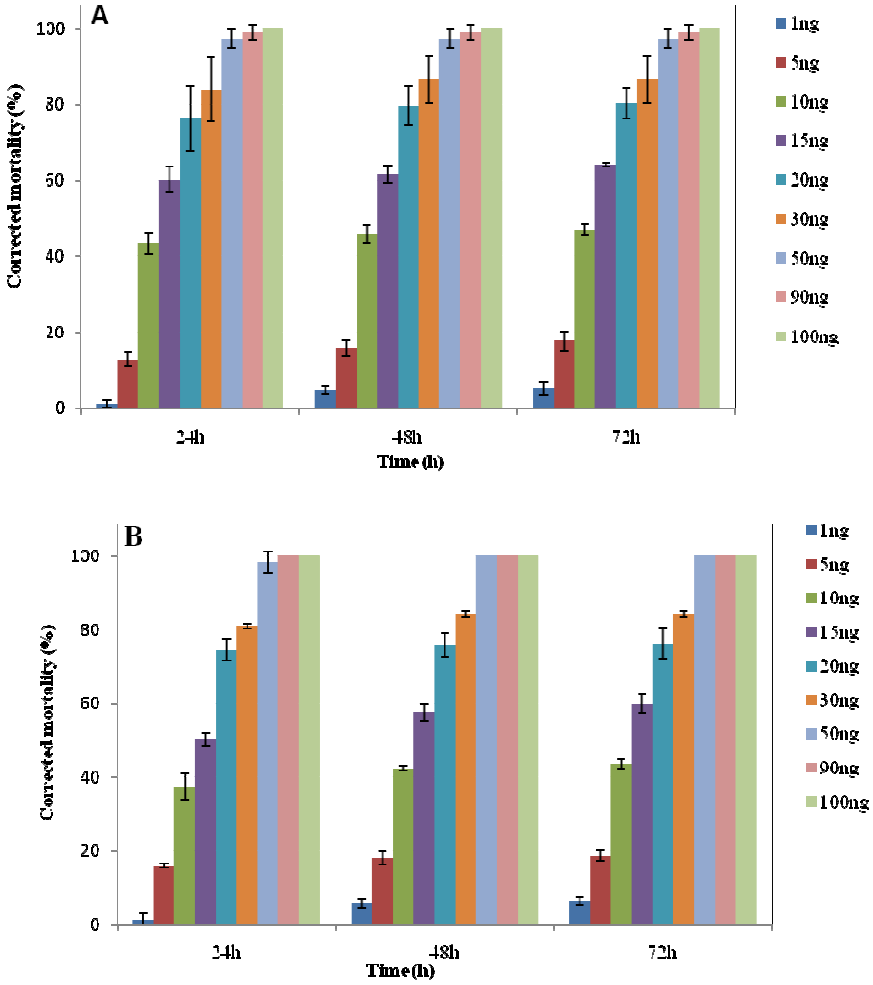



**Figure 2: Mortality Kinetics after Oral Application of Thiamethoxam**

**A :** *Apis mellifera intermissa***; B :** *Apis mellifera sahariens is***.** Bee mortality was monitored after ingestion of thiamethoxam at the doses of 1ng, 5ng, 10ng, 15ng, 20ng, 30ng, 50ng, 90ng and 100 ng /abeilles. Data represented the means ± SD of three experiments performed in triplicate. The absence of error bars corresponds to SD = 0.

**Contact Toxicicty**

The results of acute toxicity by topical application are presented in Figure 3A and B. Mortality increases according to the dose used for the both subspecies. In fact, mortality is not delayed and the maximum mortality is reached 24 hours after topical application of thiamethoxam. This phenomenon is much more pronounced for *A. m. intermissa*, 100% mortality after 24 h (Figure 3A). By cons for *A. m. sahariensis*, 100% mortality are generated with a higher dose of 200 ng / bee and after a longer duration (48 hours) (Figure 3B). But for a higher dose, 250 ng / bee 100% mortality occur after 24 hours. A low dose of 10 ng / bee produces low mortality after 24 hours Tellian bee (7.4%) and the Saharan bee (11%). The mortality of *A. m. sahariensis* increases reaching 11.9% at 48 and 72 hours. For *A. m. intermissa*, mortality rates observed at doses of 50 and 100 ng / bee are very high reaching 81 and 96% respectively, while for *A. m. sahariensis*, these same doses generate lower mortality rate is 48% and 77%. The results of ANOVA analysis ($p < 0.0001$) showed a highly significant treatment effect as regards the sensitivity to thiamethoxam for both honeybees subspecies to different observation times.

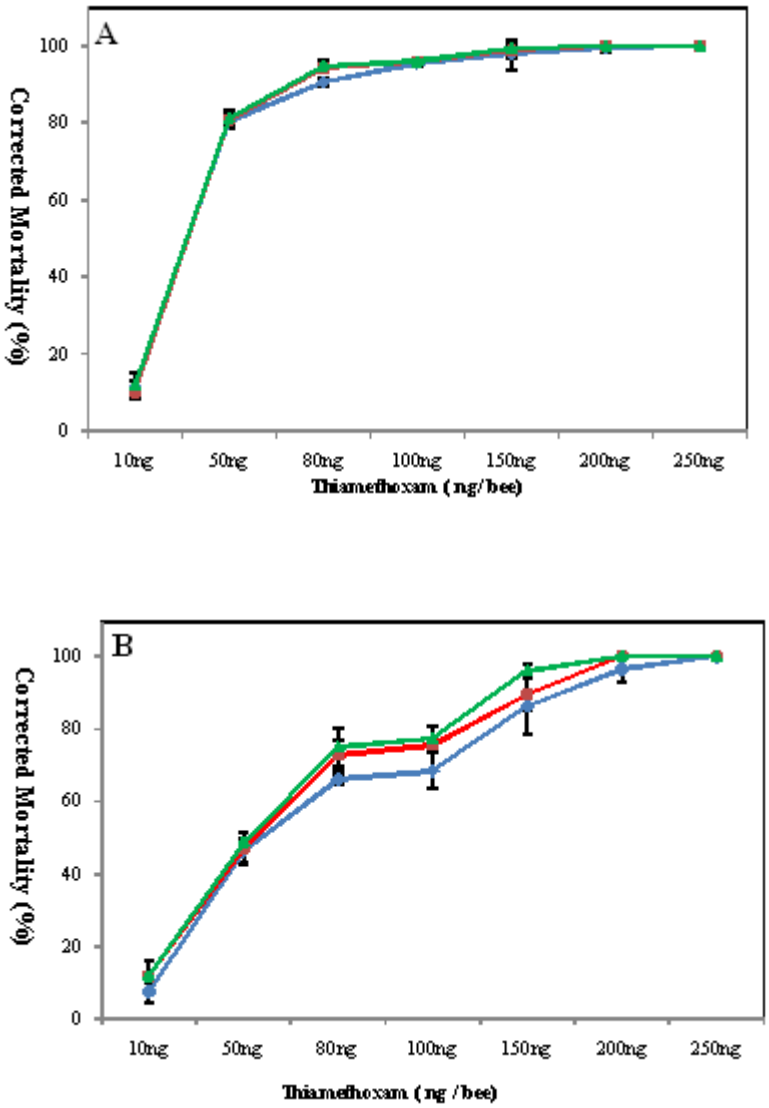



**Figure 3: Dose Response Relation Resulting from Contact Exposure to Thiamethoxam**

**A :** *Apis mellifera intermissa***; B :** *Apis mellifera sahariensis***.** Bee mortality observed 24, 48 h and 72 h after oral application of different thiamethoxam doses. Data represented the means ± SD of three experiments performed in triplicate. The absence of error bars corresponds to SD = 0

**Kinetics of Mortality**

The results of the kinetics of mortality are shown in Figure 4 A and B. The aspect of this is classic kinetics. The higher the dose of thiamethoxam increases, mortality rises. In *A. m. intermissa* maximum mortality is reached 24 hours after topical application to thiamethoxam (Figure 4A), whereas for *A. m. sahariensis* maximum mortality is reached after 48 hours (Figure 4B). It is noteworthy that 100% mortality are reached for both subspecies after 48 hours when they are subjected to high doses.

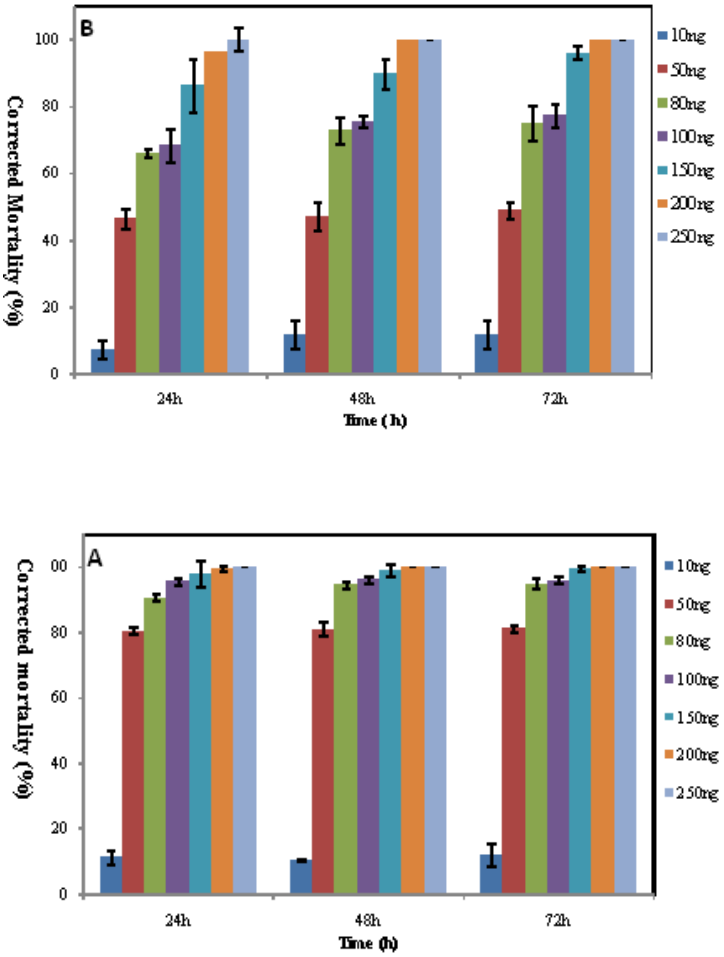



**Figure 4: Mortality Kinetics after Oral Application of Thiamethoxam**

**A:** *Apis mellifera intermissa***; B:** *Apis mellifera sahariensis***.** Bee mortality was monitored after ingestion of thiamethoxam at the doses of 10ng, 50ng, 80ng, 100ng, 150ng, 200ng and 250ng /bee. Data represented the means ± SD of three experiments performed in triplicate. The absence of error bars corresponds to SD = 0.

**LD50 Values of Thiamethoxam**

The LD50 values of thiamethoxam in honeybee species obtained with contact and oral tests are summarized in Table 1. LD50 values obtained for thiamethoxam honeybees are low. Indeed, for *A. m. intermissa*, average values LD50 at 24 hours are respectively about 12,3 and 26 ng / bee for oral and contact applications (Table 1). For *A. m. sahariensis*, average values LD50 at 24 hours are respectively about 13,3 and 43,3 ng / bee for oral and contact applications. The LD50 values are identical for both honeybee subspecies after oral administration at 48 and 72 hours. For *A. m. sahariensis*, the LD50 values after contact application are high compared to those obtained with *A. m. intermissa*. The ANOVA analysis ($p < 0.0001$) showed a significant difference in sensitivity between the thiamethoxam for both honeybee subspecies after topical application.

**Tableau 1 -** LD 50 values of thiamethoxam in *Apis mellifera intermissa* et *Apis mellifera sahariensis*. The LD50 values were obtained from the experiments carried out with different thiamethoxam doses either after oral or contact

application. The LD50 values were determined from regression lines obtained by Probit transformations of the percentages of mortality corrected, and changes in logarithm of doses. Values in brackets represented 95% confidence limits.

**Table 1**

| | **LD50 (ng/abeille)** | | | |
|---|---|---|---|---|
| | ***A. m. Intermissa*** | | ***A. m. Sahariensis*** | |
| | **Oral** | **Contact** | **Oral** | **Contact** |
| **24h** | **12,29** [10,22 – 14.36] | **26,01** [22,01 – 30,01] | **13,34** [11,26-15,40] | **44,33** [40,33 – 48,33] |
| **48h** | **11,12** [9,05 – 13,19] | **25,39** [21,53 – 29,52] | **11,76** [9,68 – 13,82] | **39,51** [35,51 – 43,51] |
| **72h** | **10,64** [8,37 – 12,51] | **24,33** [20,33 – 28,33] | **10,82** [8,75 – 12,89] | **36,84** [32,84 – 40,84] |

## DISCUSSIONS

### Symptoms Induced by Thiamethoxam

Oral toxicities and contact quickly induce symptoms of neurotoxicity such as disorderly movements, tremors and convulsions, and apathetic behavior. The same symptoms are induced by imidacloprid in *Apis mellifera mellifera* and *Apis mellifera caucasica* (Suchail, 2001; Suchail *et al.*, 2003). Neonicotinoid insecticides act as nerve agents and affect the mobility of bees inducing symptoms such as tremors, uncoordinated movements and hyperactivity (Lambin *et al*., 2000; Nauen *et al*, 2001; Suchail *et al*. , 2000, 2003; Medrzycki *et al*, 2003; Colin *et al*, 2004). These symptoms are easily observed at high levels of exposure, whereas the effect of a lower dose is difficult to discern.

### Oral and contact toxicity

Thiamethoxam is more toxic orally than by topical application, which is different from the action of most insecticides used. Similar findings are reported by Suchail *et al*. (2000) with imidacloprid. By against, other insecticides on bee exhibit greater toxicity after topical application after ingestion. Topical application of a synthetic pyrethroid, deltamethrin, toxicity generates 16 times higher than that observed after ingestion (Smart and Stevenson, 1982). Similarly, the toxicity of organophosphates such as chlorpyrifos, contact appears to be higher than oral (Mishra and Verma, 1982) four times. Rafalimanana (2003) observed after the use of chlorpyrifos, deltamethrin and lambda cyhalothrin in the laboratory that the honeybee is more sensitive to these insecticides by topical application as collective ingestion. Gilbret and Wilkinson (1975) cited by Rafalimanana (2003) reported that the ingested product spends in detoxification organs or gut and Malpighian tubules before being distributed throughout the body. As against the product applied to the thorax through the cuticle through waxy canaliculi and distribution is carried out directly in the body, especially in the more lipophilic areas. Generally, neonicotinoids are more toxic orally by contact mode. The difference between oral and contact toxicity may be due to the low hydrophobicity of neonicotinoids producing low penetration through the insect cuticle (Decourtye and Devillers, 2010). It is to note that the maximum mortality is reached after 24 hours of treatment, the dose-mortality curves are superimposed relationship. This feature of the toxicity of thiamethoxam suggests the involvement of metabolic phenomena. Indeed, the metabolism of thiamethoxam is widely studied in *Rhizobacterium Ensifer adhaerens*, mice, insects and plants such as cotton, spinach and tomato. In these organisms, thiamethoxam initially undergoes processing by one of three ways: either to demethylation desmethyl thiamethoxam, or the reduction of nitro to nitrosoimino (= N-NO) followed by conversion to the imino (= NH) and urea (= O) for thiamethoxam and metabolites desmethyl-thiamethoxam, or is the cleavage of oxadiazine cycle that will give clothianidin, a neonicotinoid insecticide newly marketed (Nauen *et al*, 2003; Ford and Casida, 2006, 2008; Karmakar *et al*, 2009. Casida, 2011; Zhou *et al*, 2012). Nauen *et al*. (2003) reported that thiamethoxam is metabolized in the body of the insect that clothianidin is highly toxic insecticide to bees. Indeed, these authors note that the N - desmethyl - thiamethoxam is not significantly produced in the caterpillars of

*Lepidoptera Heliothis virescens* and *Spodoptera frugiperda* or in cotton, although it is often mentioned as a possible metabolite. These authors also conclude that thiamethoxam is likely to be a precursor of neonicotinoids for clothianidin. It is quite possible that in the present toxicity tests, cumulative mortality at 24 hours after treatment is due to clothianidin. A similarly high toxicity of imidacloprid and thiamethoxam is also observed in the bumblebee *Bombus terrestris* (Mommaerts *et al*., 2010). When topical treatment, containing nitro - groups as neonicotinoids imidacloprid, clothianidin, thiamethoxam, dinotefuran and nitenpyram are more toxic than the cyano group - containing acetamiprid and thiacloprid (Iwasa *et al,* 2004; Laurino *et al*., 2011). The low toxicity of neonicotinoids cyano groups can be attributed to their rapid biotransformation (Suchail *et al*, 2004a, b;. Brunet *et al*, 2005)

**Oral and contact LD50**

The LD 50 values obtained confirm that thiamethoxam is more toxic by oral administration than by topical application. Iwasa *et al*. (2004) classify the toxicity of neonicotinoid insecticides depending of LD50 obtained at 24 hours after topical application. For the nitro group, the LD50 obtained are: imidacloprid (18 ng / bee) > clothianidin (22 ng / bee) > thiamethoxam (30 ng / bee) > dinotefuran (75 ng / bee) > nitenpyram (138 ng / bee) and those obtained for the cyano group are : acetamiprid (7µg/bee) > thiacloprid (15µg/bee). Suchail *et al*. (2000) note that LD50 obtained with imidclopride for oral applications and two contact are between 4 and 24 ng / bee. Decourtye and Devillers, (2010) reported that the LD50 values of imidacloprid topically does not significantly vary from one author to another, the change can be multiplied by a factor of 6. Using the same route of application, Stark *et al*. (1995) show that the three species of bees or *Apis mellifera* , *Megachile rotundata* and *Nomia melanderi*, are also susceptible to imidacloprid (LD50 = 0.04 mg / bee after 24 h)

Similar conclusions are drawn for thiamethoxam with an LD50 of 30 ng / bee for *A. mellifera* and 33 ng / bee for *B. terrestris* (Iwasa *et al*, 2004; Mommaerts *et al*, 2010.). The LD50 values obtained in this study for *A. m. sahariensis* topically are higher than those reported in the literature. By against, those mentioned for *A. m. intermissa* are lower. Moreover, the values obtained by ingestion are higher compared to those noted by several authors (Decourtye and Devillers 2010; Laurino *et al*, 2011.). It is found that the oral LD50, show great variability in different studies with neonicotinoids (Decourtye and Devillers 2010; Laurino *et al*, 2011.). Thus, the oral LD50 of imidacloprid can vary from 1 to 20 times. This variation is interpreted by Nauen *et al.* (2001) by the process of trophallaxis which may contribute to differences in the absorption and accumulation of an insecticide by the workers. By against, heavy can cause a reduction in the consumption of sugar water. Rafalimanana (2003) concludes that the collective intake is closer to reality in social insects and would recommend not underestimating the toxicity. Further studies on the variation of toxicity involve other factors such as the age of the bee colony and subspecies used (Suchail *et al.*, 2000, 2001; Nauen *et al*., 2001; Guez *et al*., 2003) and bee health with an optimal protein supply (Wehling *et al*., 2009). Similarly, infestation by Nosema ceranae can make bees more susceptible (Alaux *et al*., 2010. Vidau *et al*., 2011;. Aufauvre *et al*., 2012). LD50 values of neonicotinoids are weak for honeybees compared to the oldest families of insecticides such as organophosphates, pyrethroids and carbamates which have a very high toxicity (Atkins *et al.*, 1981; STARK *et al*, 1995). Suchail *et al*. (2000) reported that the toxicity of organophosphate insecticides, such as chlorpyrifos, is four times higher contact than oral (contact LD50= act 59 ng / bee and oral= 250 ng / bee). Similarly, topical application of bifenthrin (pyrethroid ) is seven times more powerful than oral application (contact LD50 = 15 ng / bee oral , LD50 = 100 ng / bee) . Whatever breeds or species of bees used, the toxicity of thiamethoxam is extremely high. Laboratory studies and field indicate that thiamethoxam is highly toxic to bees either by contact or by ingestion (Rancan *et al*., 2006). These results confirm the potential hazard of this insecticide

should not be used in full bloom, to avoid the risk of poisoning of honeybees.

## CONCLUSIONS

The toxicity of thiamethoxam insecticide commonly used in plant protection in Algeria is characterized by the rapid onset of symptoms of neurotoxicity observed in both *A. m. intermissa* and *A. m. sahariensis* mortality occurring 15 minutes after ingestion of high doses. There is a relationship between the dose directly proportional thiamethoxam administered and mortality observed, the maximum mortality was obtained after 24 hours for high doses, phenomenon much more pronounced for *A. m. intermissa*., as *A. m. sahariensis*, after topical application. Thiamethoxam is more toxic orally than topically for both subspecies. These results confirm the potential hazard of this insecticide should not be used in full bloom, to reduce the risk of poisoning of bees.

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